भारतीय मानक
Indian Standard

IS 17433 : 2020

वस्त्र रंजक सामग्री — *टर्मिनैलिया अर्जुना* (अर्जुन) की छाल से बना प्राकृतिक रंग — पहचान की विधि

Textile Dyestuffs — Natural Dye from *Terminalia arjuna* (*Arjun*) Bark — Identification

ICS 59.040

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August 2020 **Price Group 5**

Textile Speciality Chemicals and Dyestuffs Sectional Committee, TXD 07

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by Textile Speciality Chemicals and Dyestuffs Sectional Committee had been approved by the Textile Division Council.

The revival of natural dyes has been for two reasons:

a) Due to the harmful effect of synthetic dyes, safer natural dyes are being preferred globally, and

b) Ban on the use of azo dyes which release carcinogenic amines has also brought back natural dyes in limelight. Natural dyes are being used extensively for dyeing of cotton, linen, silk and wool substrates.

Terminalia arjuna (Arjuna, *Arjun* in Hindi) bark has been variously used for medicinal purposes in improving cardio vascular health, and for tanning and dyeing purposes. It has been used for dyeing textiles in shades of brown since ancient times.

In order to achieve optimum quality with minimum lot-to-lot shade variation, and little differences in colour when the same dyestuff is obtained from different manufacturers, it is essential to standardize this natural dyestuff. This will improve the cost benefit ratio and help the user adjust their dyeing or printing recipes appropriately.

It is for the first time that the standard for natural dyestuff *Terminalia arjuna* (*Arjun*) bark dye has been written, it is mainly for the ease of buyers, seller and users of this natural dyestuff. Standardization and identification methods will bring all the major parameters under scan and match. The main intention of this specification is to identify the natural dyestuff

The composition of the Committee responsible for the formulation of this standard is given in Annex F.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’.

Indian Standard

TEXTILE DYESTUFFS — NATURAL DYE FROM *TERMINALIA ARJUNA* (*ARJUN*) BARK — IDENTIFICATION

1 SCOPE

This standard prescribes test method and identification requirement of powdere/ aqueous extract of *Terminalia arjuna* (*Arjun*) bark dye, and of textiles coloured with this dye. The detection and identification of the natural colourant from *Terminalia arjuna* (*Arjun*) bark is conducted using Kit method given in Annex A, chromatographic techniques, that is Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) as given in Annex B and C and by UV-Visible spectroscopic technique as given in Annex D. The method for extraction of the dye from textile dyed with *Terminalia arjuna* (*Arjun*) bark extract is given in Annex E.

2 REFERENCE

The following standard contain provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated was valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below.

IS No.	*Title*
1070 : 1992	Reagent grade water – Specification (*third revision*)

3 TERMS AND DEFINITIONS

For the purposes of this standard, the following terms and definitions apply:

3.1 *Terminalia arjuna* (*Arjun*) Dye — The botanical name of Arjun tree is *Terminalia arjuna,* it belongs to family Combretaceae. *Terminalia arjuna* bark is used variously as medicine for heart problems and high blood pressure, astringent, tannin source, and dye. It has been used for dyeing textiles in shades of brown since ancient times.

3.2 Natural Colourants — Colourants from natural origin, particularly materials obtained from plant parts, namely - stem, root and leaves, extracted without any chemical reaction primarily used in textiles for dyeing or printing.

4 PRINCIPLE

Natural colorants are usually never a single compound, they are composed of several compounds many of which are structurally similar and therefore visual response to one or more common laboratory chemicals may give a preliminary indication about the presence of those specific type of compounds found in a particular natural dye. The individual character of different compounds present in a natural colorant will determine how they bind to the textile substrate, and the ratios bound to the substrate may be different from their ratios in the extract. These individual components can be analyzed out by spectroscopic and chromatographic techniques which can establish the authenticity of the natural colourant. *Terminalia arjuna* bark is rich in tannins and flavonoids which contribute to the chromatographic and spectroscopic characteristics of its extract.

5 APPARATUS

5.1 Analytical Balance, with resolution of 0.001 g.

5.2 Spatula

5.3 Ultrasonic Water Bath (Sonicator)

5.4 Stoppered Conical Flask

5.5 Measuring Cylinder

5.6 Funnel

5.7 Qualitative Grade Filter Paper — Particle retention 11 µm.

6 REAGENTS

6.1 *Terminalia arjuna* Bark, dried and powdered to serve as reference.

6.2 Methanol — Analytical grade.

7 PROCEDURE

7.1 Preparation of *Terminalia arjuna* (*Arjun*) Bark Dye Reference and Test Sample

Powder made by pulverizing authentic *Terminalia arjuna* bark material is used to prepare the reference sample. The stock solution of the reference natural dye is prepared by steeping 0.5 g of dry bark powder in 100 ml methanol in a stoppered conical flask. It is then agitated in a sonicator set at 45°C for about 20 min after which the solution is filtered using a filter paper, and then transferred to another stoppered glass vessel for further analysis. If not used on the same day, the stock solution can be stored in a refrigerator for about two weeks. Stock solution of the test sample is also prepared in the same way from dry, powdered plant material. If the test sample is a liquid concentrate or a spray dried extract, a shorter dissolution time and a smaller amount of sample (0.05 g) may be sufficient. If the test material is dyed textile, the method for the extraction of the natural dye from it is given in Annex E.

7.2 Analysis

The detection and identification of natural *Terminalia arjuna* (*Arjun*) dye from prepared dye samples is conducted using Kit method given in Annex A, by using chromatographic techniques viz., TLC and HPLC as given in Annex B and C as well as by UV-Visible spectroscopy as given in Annex D.

7.3 Qualification and identification of *Terminalia arjuna* (*Arjun*) Natural Dye

Comparison between analyses of test sample and reference *Terminalia arjuna* dye sample through **7.3** can identify whether the test sample is natural *Terminalia arjuna* dye. The specific tests to be conducted are given in Table 1.

8 CALIBRATION OF THE ANALYTICAL EQUIPMENT

The instruments/equipment being used (TLC, HPLC, UV-Visible spectrophotometer) should be calibrated using standard reference materials and procedures as recommended by the manufacturer to ensure their proper working before start of the analysis.

9 VALIDATION OF THE METHOD

The test methods employed should be validated using authentic reference dye sample for reliability and repeatability

10 REPORT

The report shall include the following information:

a) Reference to this standard,
b) Tests conducted and test conditions, and
c) Results on identification of the test sample.

Table 1 Specific Tests Recommended for Identification of *Terminalia arjuna* Bark Dye

(*Clause* 7.3)

Sl No.	Test	Type of Test	Ref to Annex
(1)	(2)	(3)	(4)
i)	Kit test	Preliminary test	A
ii)	Chromatography tests		
	a) Thin layer chromatography (TLC)	Mandatory test	B
	b) High performance liquid test chromatography (HPLC)	Additional (*See* Note)	C
iii)	Spectroscopy tests		
	a) UV-visible spectroscopy	Confirmatory test	D

NOTE— HPLC may be performed wherever such instrumentation facilities are available.

ANNEX A

(*Clauses* 1, 7.2 *and Table* 3)

KIT TEST FOR IDENTIFICATION OF *TERMINALIA ARJUNA* (*ARJUN*) BARK DYE

A-1 PRINCIPLE

Each natural dye has its characteristic molecular composition which is the distinctive feature of that dyestuff. The *Terminalia arjuna* (*Arjun*) dye gives a characteristic reaction with sodium hydroxide solution which is used for its identification.

A-2 APPARATUS

A-2.1 Test Tubes

A-2.2 Test Tube Holder

A-2.3 *p*H Meter

A-2.4 Pipette

A-2.5 Measuring Cylinder

A-3 REAGENTS

A-3.1 Distilled Water

A-3.2 Sodium Hydroxide, Analytical Grade

A-3.3 Stock Solutions of Test and Reference *Terminalia arjuna* (*Arjun*) Dye

A-4 PROCEDURE

The stock solution of both the reference and test *Terminalia arjuna* (*Arjun*) dye is diluted 10 times with water and 10 ml of the diluted solution is taken in a test tube. This solution is then made alkaline (*p*H 10.6) by using 10 percent solution of sodium hydroxide (NaOH) and observed for any colour change.

A-5 OBSERVATION

A dark maroon colour develops in the solution of reference *Terminalia arjuna* dye. If a similar colour change is observed in test sample also, it is likely to be *Terminalia arjuna* dye.

A-5 CONCLUSION

The development of dark maroon color in test sample gives an indication that it is *Terminalia arjuna* dye

ANNEX B

(*Clauses* 1, 7.2 *and Table* 3)

IDENTIFICATION OF *TERMINALIA ARJUNA* BARK NATURAL DYE BY THIN LAYER CHROMATOGRAPHY (TLC)

B-1 PRINCIPLE

TLC separates non-volatile constituent compounds in a mixture by virtue of differences in their affinity for the stationary solid phase which is spread as a thin layer on a sheet/plate and the selected mobile solvent phase which are then seen as different spots on the TLC plate. Positioning of a spot on the TLC plate denoted by its R_f value (distance travelled by the constituent / distance travelled by the solvent) depends on its chemical nature and is its characteristic property. Therefore, due to similarity in constituent chemical components, test and reference *Terminalia arjuna* dye samples under similar TLC condition should result in spots of similar R_f values and colour and thus a test sample can be identified as whether it is *Terminalia arjuna* bark natural dye.

B-2 APPARATUS

B-2.1 Analytical thickness silica gel G/ GF 254 coated TLC plates (may be cast in-house or procured from commercial suppliers)

B-2.2 Capillary for spotting TLC plates

B-2.3 TLC development chamber (of a dimension suitable to accommodate TLC plate)

B-2.4 TLC spot visualization chamber containing crystals of iodine (of a dimension suitable to accommodate TLC plate)

B-2.5 Optionally, a viewing chamber for TLC with 254 nm UV tubes which may also be fitted with daylight fluorescent tubes (CRI 95) and photographic camera for recording photographs

B-3 REAGENTS

B-3.1 Stock Solutions, of test and reference *Terminalia arjuna* bark natural dye prepared in **7.1.**

B-3.2 Methanol

B-3.3 Ethyl Acetate

B-3.4 n-Hexane

NOTE — All reagents except **B-3.1** should be of analytical reagent grade and all glassware should be cleaned and finally rinsed with reagent grade water and dried.

B-4 PROCEDURE

Both reference and the test sample extracts are spotted carefully, side-by-side, on the silica gel coated TLC plate. The spots are dried and the plate is placed in TLC development chamber, pre-equilibrated with the developing solvent consisting of 60 percent ethyl acetate and 40 percent n-hexane. After the solvent front has travelled to sufficient distance, the TLC plate is removed and the solvent is allowed to evaporate. Developed spots are visualized in daylight. The spots may optionally be visualized and photographed in the viewing chamber under daylight fluorescent tubes and if a silica gel GF254 plate is used, also under UV-254 nm light. The TLC plate is then placed in the iodine chamber and the spots are visualized in iodine. The constituents of the test sample present as spots on the TLC plate are compared with the reference dye sample. The colour and R_f values of the spots in both the test and reference sample are noted.

B-5 OBSERVATION

Details of stock solution preparation and its dilution if any,

Type of TLC plate used: Silica gel G/ GF 254; in-house/ commercial

Solvent system used: 80 percent ethyl acetate and 20 percent n-hexane

R_f and spot colour in reference sample of *Terminalia arjuna* bark dye	R_f and spot colour in test sample	Comment
R_f 0.60 major and 0.20 minor		

An indicative thin layer chromatogram with both the tracks being natural *Terminalia arjuna* bark dye is presented in Fig. 1. However, the position and number of spots may vary due to differences in sample preparation and chromatographic conditions.

A-6 CONCLUSION

Colour of the spots and the R_f values of the reference and test samples are similar/different.

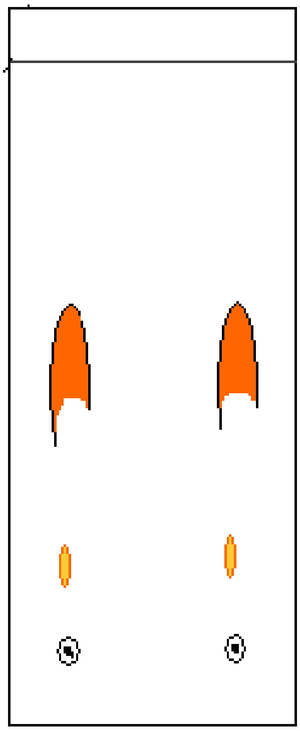

FIG. 1 THIN LAYER CHROMATOGRAM OF *TERMINALIA ARJUNA* BARK DYE

ANNEX C

(*Clauses* 1, 7.2 *and Table* 3)

IDENTIFICATION OF *TERMINALIA ARJUNA* BARK DYE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

C-1 PRINCIPLE

HPLC is used to separate compounds in a mixture by virtue of differences in their affinity for the stationary phase which is packed in a column, and the mobile solvent phase that is pumped through it. The constituents, depending upon their chemical nature, are differently retarded during their passage through the column and are identified by comparing the time taken by a constituent compound in eluting from the column as detected by a detector (retention time) with that of a standard reference compound. Selection of a detector is important as in the closed system, the eluting compounds cannot be seen by the eye and can only be detected by their characteristic properties. UV detector is a common detector employed as many organic compounds absorb in UV region. Wavelength of the detector can be set to a value where absorption by the compounds of interest is high so as to achieve higher sensitivity. Various parameters of the HPLC assay are set in such a way that distinct peaks of the reference sample are observed in the chromatogram.

C-2 APPARATUS

C-2.1 HPLC System — with binary pump, UV/PDA detector and software

C-2.2 C-18 Reverse Phase Column (RPC-C18) — 150 x 4.6 mm; 5 micron.

C-2.3 Guard Column, compatible with the analytical HPLC column .

C-2.4 Micro-Syringe

C-2.5 Membrane Filters (0.45 micron), for micro -syringe.

C-2.6 All Glass Filtration Assembly, for micro filtering solvents.

C- 2.7 Oil-Free Vacuum Pump

C-2.8 Membrane Filters (0.45 micron), for solvent filtration.

C-3 REAGENTS

C-3.1 Methanol

C-3.2 Deionized Water

C-3.3 Reference *Terminalia arjuna* bark natural dye and test dye stock solutions prepared in **7.1** after appropriate dilution with methanol

NOTE — All reagents except **C-3.3** should be of HPLC grade and need to be filtered through 0.45 micron membrane filter before use. All glassware used should be cleaned and finally rinsed with reagent grade water and dried.

C-4 PROCEDURE

C-4.1 Chromatographic conditions

a)	Stationary phase:	Column C18, 150 × 4.6 mm; 5 micron,
b)	Eluent/ mobile phase : Methanol :	Deionised water (95:5),
c)	Flow rate:	1.0 ml/min,
d)	Detection wavelength:	254 nm (band width 16 nm), and
e)	Run time:	15 min.

C-4.2 Method

C-4.2.1 HPLC system is started and the selected mobile phase is allowed to pass through the column till a stable baseline is obtained. Reference dye solution is then aspirated into the micro-syringe, a membrane pre-filter is fitted onto it and the solution is injected into the sample loop (10-20 micro litre capacity) in load position to fill it completely. The injection valve is then turned to inject position to introduce the sample onto the column. As the mobile phase is continuously passing through the column, the constituents of the injected dye extract get separated. These separated constituents exit the column and enter the UV/PDA Detector which detects them by recording the absorbance of the individual constituents as they pass through it. This absorbance is converted to a signal and a peak is seen on the signal intensity versus time plot continuously displayed on the monitor. When a UV absorbing component passes through the detector, a peak is seen on the plot. After completion of the run, a chromatogram along with a report of peak retention times, peak height and peak area for each peak is generated by the software. The test sample run is also completed in the similar manner. Identity of the test sample is established by comparing the retention times of the peaks in reference and test chromatograms. Peak height or its area provides additional quantitative information about the relative abundance of various constituents in test and reference samples.

C-4.2.2 An indicative chromatogram containing the peaks obtained after HPLC assay of the *Terminalia arjuna* bark natural dye extract is shown in Fig. 2. Peak location/retention times and height may vary between the indicative chromatogram and the injected samples due to differences of the dye sample and the extract preparation conditions.

C-5 OBSERVATION

	In Reference Terminalia. *arjuna* bark natural dye	In Test Sample	Comment
Retention time of the peaks observed in HPLC chromatogram	1.8 min 3.0 min 3.3 min		

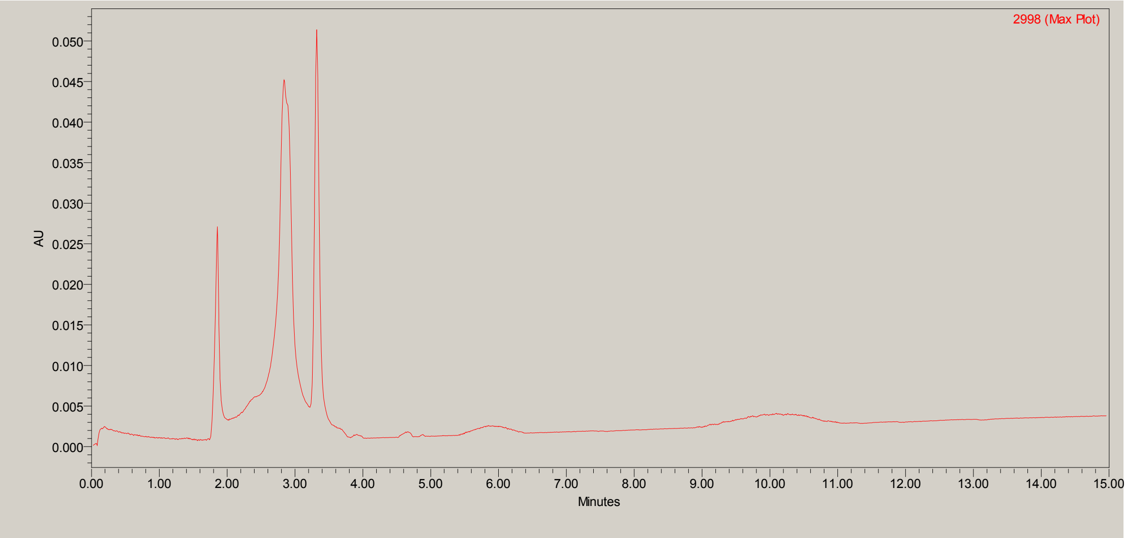

FIG. 2 CHROMATOGRAM OF THE *TERMINALIA ARJUNA* BARK DYE

C-6 CONCLUSION

The retention time of the constituents of the reference and test sample are similar/different. The reference sample and test sample are therefore similar/different.

ANNEX D

(*Clauses* 1, 7.2 *and Table* 3)

IDENTIFICATION OF *TERMINALIA ARJUNA* BARK DYE BY UV — VISIBLE SPECTROSCOPIC METHOD

D-1 PRINCIPLE

When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some of the light energy will be absorbed as the electron is promoted to a higher energy orbital. An optical spectrometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength. The resulting spectrum is presented as a graph of absorbance (A) *versus* wavelength.

Many molecules absorb ultraviolet and/or visible light. Fine particles will scatter light and contribute to the absorbance. It is therefore essential to remove fine particles when measuring absorbance of dye solutions. Absorbance (A) is directly proportional to the path length, b, and the concentration, c, of the absorbing species. *Beer's law* states that

$$A = \varepsilon bc$$

Where, ε is a constant of proportionality, called the absorptivity.

Different dye compounds within the natural dyestuff absorb radiations of different specific wavelengths. The spectral curve will thus be a summation of their individual absorbances. An absorption spectrum of the natural dyestuff will thus show a characteristic curve with peaks corresponding to structural groups present within the constituent molecules.

D-2 APPARATUS

D-2.1 Ultra Violet Visible Scanning Spectrophotometer

D-2.2 Quartz Sample Cuvettes — Matched pair of 1.00 cm light path.

D-3 REAGENTS

D-3.1 Reference *Terminalia arjuna* Bark Dye Extract

D-3.2 Methanol

NOTE — All reagents should be of analytical reagent grade and all glassware should be cleaned and finally rinsed with reagent grade water and dried.

D- 4 PROCEDURE

D-4.1 The stock solution of the reference *Terminalia arjuna* bark dye prepared in **7.1** may be used to record the spectrum in the UV-visible region (200–700 nm). If the absorbance in the UV region is too high, this solution may be suitably diluted for recording the spectrum in the UV region (200–400 nm). The test sample solution is also prepared in the same manner as the reference sample.

D-4.2 Both visible and UV spectra are recorded with pure methanol in reference holder of the spectrophotometer. Reference dye sample is first analysed. Test sample is analysed next and recorded spectra are compared for similarity of the spectral curve that is, peak positions and their intensity (absorbance value). The absorbance spectral curve of this dye extract has specific characteristics both in the UV and visible range. An indicative UV spectrum of the *Terminalia arjuna* leaf dye extract is presented in Fig. 3.

D-5 OBSERVATION

D-6 CONCLUSION

The nature of the spectral curve of the test sample in the UV and visible range is similar/different to the reference sample.

	In reference *Terminalia arjuna* bark extracts	**In test extract**	**Comments (Describing difference/ similarity in spectral curve)**
Peak position (nm) with absorbance	232 nm — 1.56 A 278 nm — 1.70 A 364 nm — 0.61A 410 mm — 0.31A		

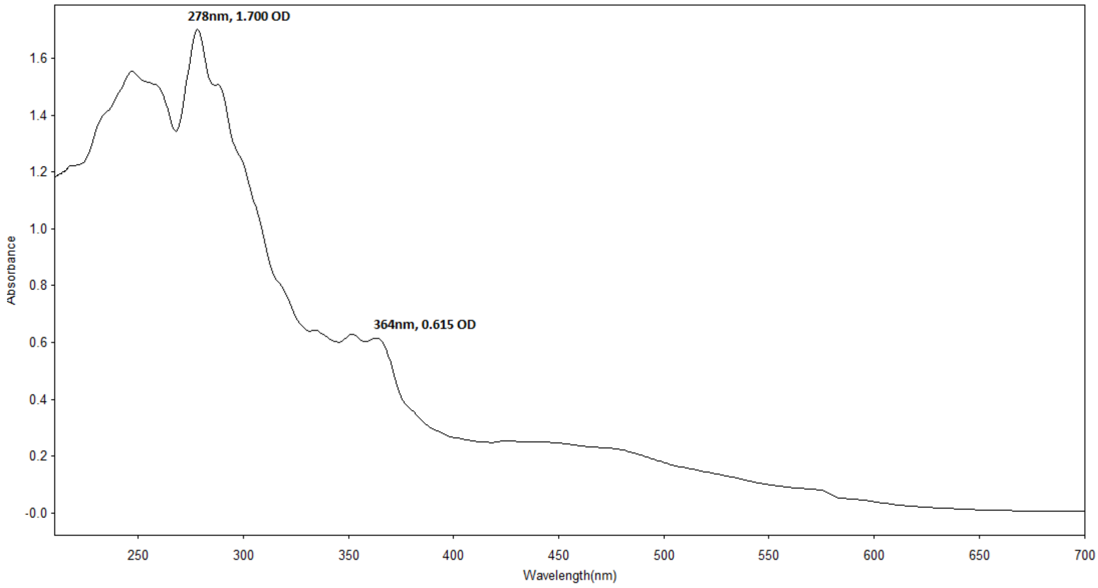

FIG. 3 UV - VISIBLE SPECTRUM OF THE *TERMINALIA ARJUNA* BARK DYE

ANNEX E

(*Clauses* 1 *and* 7.1)

EXTRACTION OF DYE FROM FABRIC/YARN DYED WITH *TERMINALIA ARJUNA* BARK DYE

E-1 PRINCIPLE

Extraction of dyed textile samples with a suitable solvent (methanol) is necessary to release the dyes from these samples.

E-2 APPARATUS

E-2.1 Soxhlet Extraction Assembly with Heating Bench

E-2.2 Scissors

E-2.3 Qualitative Filter Paper Sheet

E-2.4 Rotary Vacuum Evaporator with Evaporating Flask

E-2.5 Funnel

E-2.6 Conical Flasks

E-2.7 Measuring Cylinder

E-3 REAGENTS

E-3.1 Methanol (Analytical Reagent Grade)

E-4 PROCEDURE

About 50 g of dyed textile is cut into small pieces and packed in a filter paper. The packed filter paper column is put for soxhlet extraction with methanol as the solvent. The temperature is so adjusted to get about 8-10 cycles of the solvent per hour. Extraction is continued for 6-8 h, till most of the colour is discharged. The coloured solvent is filtered and is concentrated under vacuum in a rotary evaporator to about 20 ml. This concentrated extract can then be used for chromatographic and spectroscopic analysis as per the methods described in **7.3** after appropriate dilution, if required.

ANNEX F

(*Foreword*)

COMMITTEE COMPOSITION

Textile Speciality Chemicals and Dyestuffs Sectional Committee, TXD 07

Organization	*Representative(s)*
Department for Jute and Fibre Technology, Institute of Jute Technology, University of Calcutta	Prof A. K. Samanta (***Chairman***)
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Ama Herbals, Lucknow	Shri Y. A. Shah
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Indian Jute Industries Research Association, Kolkata	Dr S. K. Chakrabarti Shri Sandip Basu (*Alternate*)
Jaysynth Dye-Chem, Mumbai	Shri S. C. Sharma Shri P. M. Reshamwala (*Alternate*)
National Peroxide Limited, Mumbai	Shri K. H. Deshpande Shri V. N. Joshi (*Alternate*)
Northern India Textile Research Association Ghaziabad	Dr M. S. Parmar
Office of the Textile Commissioner Mumbai	Shri Humayun K. Shri Jamil Ahmed (*Alternate*)
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The South India Textile Research Association, Coimbatore	Dr Prakash Vasudevan Shri S. Sivakumar (*Alternate*)
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U P Textile Technology Institute, Kanpur	Prof (Shrimati) Abha Bhargava Dr Arun Patra (*Alternate*)

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SHRI A. K. BERA
SCIENTIST 'F' AND HEAD (TXD), BIS

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Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards: Monthly Additions'.

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Amendments Issued Since Publication

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